CHAPTER 3

Seroprevalence of Anti-HEV IgG in Swine from the Gauteng and Limpopo Provinces of South Africa

The editorial style of the *International Journal of Epidemiology* was followed in this chapter

3.1 Summary

3.1.1 Background

Hepatitis E virus (HEV) causes faecal-oral transmitted epidemic acute viral hepatitis in some developing countries. Outbreaks are predominantly associated with faecally contaminated drinking water. Hepatitis E virus may cause sporadic cases of acute hepatitis in some developed countries. The majority of these cases are, however, associated with a travel history to areas where HEV is endemic. It has been shown that some strains of HEV may be zoonotic. Swineherds in HEV endemic and non-endemic countries were found to contain pigs that are seropositive for HEV. The objective of this study was to assess the seroprevalence of anti-HEV immunoglobulin G (IgG) in selected swine populations in South Africa.

3.1.2 Methods

The study population comprised 192 swine from various regions of the Gauteng and Limpopo Provinces, South Africa. Swine serum was analysed for the presence of anti-HEV IgG by use of an enzyme-linked immunosorbent assay (ELISA).

3.1.3 Results

The prevalence of anti-HEV IgG in the Gauteng and Limpopo Provinces was 14.29% and 15.84%, respectively, with an overall prevalence of 15.10%.

3.1.4 Conclusions

The prevalence of anti-HEV IgG in swine determined in this study was in the same order as reported in HEV non-endemic countries. According to the results, HEV may be prevalent in the swine population throughout South Africa and swine may play a meaningful role as reservoirs for the virus.

Keywords

Anti-HEV IgG, hepatitis E virus, South Africa, swine, zoonotic

3.2 Introduction

Hepatitis E virus (HEV) was initially mistaken for hepatitis A virus (HAV) as these viruses share basic clinical and epidemiological properties (1). Hepatitis E virus was first discovered in the late 1970s after it had become evident that there was a hepatitis virus other than HAV and hepatitis B virus (2). Hepatitis E virus was initially classified in the family Caliciviridae, but is currently recognised as the type species of the genus Hepatitis E-like viruses (3, 4).

Hepatitis E virus is an important public health concern in some developing countries, where it causes faecal-oral transmitted epidemic acute viral hepatitis (5-7). Hepatitis E outbreaks, primarily associated with faecally contaminated drinking water, have been reported in countries such as India, Nepal, Burma, Pakistan, Afghanistan, Borneo, China, Mexico, Egypt, Algeria, Ethiopia, Somalia, Sudan, the Ivory Coast, Botswana and Namibia (5, 7-12).

Sporadic cases of HEV have been reported in some developed countries (5, 6, 13). The majority of these cases were probably imported because they had a travel history to countries where the disease is endemic (5, 6, 13). Rare cases of sporadic hepatitis E have been reported without a history of travel, in which case the transmission of the virus remains uncertain (5, 14-17). It has, however, been established that the genomic

sequences of the HEV strains detected in these patients were more related to the swine HEV strains prevalent in the swine population of the same area than to the human HEV strains (18). These swine HEV strains may undergo genetic reversion to HEV strains which cause clinical disease in humans similar to that in many parts of the world where hepatitis E is endemic (19, 20).

Although a reservoir of HEV has not yet been established, recent findings have shown that at least some strains of HEV may be zoonotic (21-27). In support of this view, Drobeniuc et al. (2001) established that there is an increase in HEV infection among persons with occupational exposure to swine, which suggests animal-to-human transmission of HEV.

It has been reported that anti-HEV IgG was detected in swine populations from developing countries such as Nepal, China, and Thailand, as well as from developed countries such as the US, Canada, Australia, and Spain (20, 29-33). Swine herds in both HEV endemic and non-endemic countries were found to contain many pigs that are seropositive for HEV, which suggests that the virus may be enzootic in swine regardless of whether HEV is endemic in the human population (32).

In South Africa clinical cases of hepatitis E are rarely seen. Most of those on record are imported cases. However, HEV seems to be endemic in certain areas in the country because seroprevalence studies revealed that up to 15% of individuals in certain developing communities are anti-HEV positive (34). The seroprevalence of anti-HEV IgG in swine has not yet been investigated in South Africa. The objective of this study was to assess the seroprevalence of anti-HEV IgG in selected swine populations in the Gauteng and Limpopo Provinces, which may cast light on the endemic presence of the virus in the country.

3.3 Materials and Methods

3.3.1 Swine Serum Samples

Swine serum samples were collected from swine of various ages at the Agricultural Research Council in Irene, the experimental farm and Faculty of Veterinary Sciences of the University of Pretoria, and selected abattoirs in the Gauteng and Limpopo Provinces, South Africa. The blood samples (5 ml) were centrifuged (Sorvall® Super T 21) at 1 000 × *g* for 10 min at room temperature (23°C) to obtain clear non-haemolysed specimens and stored at -70°C until the samples were processed.

3.3.2 Detection of Anti-HEV IgG in Swine Serum

Anti-HEV IgG was detected by ELISA, using a mosaic protein, composed of recombinant proteins from immunoreactive epitopes of the HEV open reading frames (ORF) 2 and 3 as the target antigen (35, 36). The ELISA protocol was based on the procedure previously described (36). Immulon 2HB Styrene ELISA wells (Dynatech Laboratories Inc., Chantilly, VA) were adsorbed with 100 µl of phosphate buffered saline (PBS) (0.01 M, pH 7.2-7.4) containing 15 ng of target antigen (HEVAg) (CDC, Atlanta, GA) and incubated overnight at 4°C. Each specimen was diluted 1:10 in first specimen diluent (FSD) (CDC) in non-coated wells (Evergreen Scientific Inc., Los Angeles, CA) and incubated overnight at 4°C. Each well of the HEVAg-coated Immulon 2HB plate was washed 5 times with 200 µl 1× wash solution (CDC). Ninety microlitres of second specimen diluent was dispensed to each well of the HEVAg-coated plate, followed by the addition of 10 µl of negative control (NC), positive control (PC) and specimens, pre-diluted 1:10 in FSD, to the appropriate wells according to the ELISA protocol to attain a final dilution factor of 1:100. The plate was sealed and incubated for 45 min at 37°C. During the incubation period the rabbit anti-swine horseradish peroxidase IgG conjugate was diluted 1:3000 in conjugate diluent. After the incubation each well was washed 5 times with 1× wash solution to remove any unbound antibody, followed by the addition of 100 µl of conjugate working solution to each well. The plate was sealed and incubated for 45 min at 37°C. Each well was washed 6 times with 1× wash solution, followed by

the addition of 100 µl of substrate solution (*o*-phenylenediamine dihydrochloride) (CDC). The plate was incubated avoiding strong light for 10 min at room temperature (23°C) for colour development. The enzyme reaction was stopped by the addition of 50 µl of 1 N sulphuric acid. The optical density (OD) of the substrate solution in each well was read using an enzyme immuno assay reader (Titertek® Multiskan Plus MkII) at an absorbency of 492 nm with a reference filter of 630 nm.

The standard NC was tested four times and the standard PC was tested once for each assay run. The OD value of the PC had to be ≥0.600, the OD value of individual NCs had to be ≤0.120, and the OD value of the substrate blank had to be ≤0.040 for the assay results to be considered valid. The cut-off (CO) value was calculated as the mean NC OD + 0.300. Serum specimens with an OD value below the CO value were considered non-reactive, while serum specimens with an OD value equal to or greater than the CO value were considered reactive. Reactive specimens were re-tested in duplicate to be considered positive for anti-HEV IgG.

3.3.3 Statistical Analysis

Each pig was classified as seropositive or seronegative after the ELISA based on the criteria described above. The exact 95% confidence intervals (CI) were calculated as appropriate, using the Smith's Statistical Package version 2.5 (2001).

3.4 Results

Swine serum specimens were randomly obtained from 192 pigs from different regions in the Gauteng and Limpopo Provinces, South Africa. The overall prevalence of anti-HEV IgG was 15.10% (95% CI, 14.39-15.81%) in the serum specimens tested (Table 3.1). The prevalence of anti-HEV IgG in swine from the Gauteng and Limpopo Provinces was 14.29% (95% CI, 13.25-15.33%) and 15.84% (95% CI, 14.85-16.83%), respectively (Table 3.1). In addition, swine serum specimens were obtained from 132 pigs from one

farm in the Warmbaths District, Limpopo Province. The prevalence of anti-HEV IgG in these serum specimens was 28.03% (95% CI, 27.17-28.89%) (Table 3.1).

3.5 Discussion

In this investigation the prevalence of anti-HEV IgG in swine from the Gauteng and Limpopo Provinces was in the same order (15%) as that reported in HEV non-endemic countries such as Spain (25%), the US (18.3%), Canada (18.1%), and Korea (15%), and significantly lower than that reported in HEV endemic countries such as India (54.6-74.4%), Nepal (32.7%), Thailand (30.7%), and China (26.8%) (22, 29, 32, 33, 37, 38). Anti-HEV IgG was detected in swine from other provinces in South Africa (results not shown), such as Mpumalanga, North West, Kwa-Zulu Natal and Western Cape, which suggests that HEV may be prevalent in the swine population throughout the country. A significant difference in anti-HEV IgG prevalence could be demonstrated between swine from the Gauteng and Limpopo Provinces (14.29% vs 15.84%, $P = 0.0332$). This difference may be attributed to different densities and geographical conditions of the pig farms in these two provinces. The prevalence of anti-HEV IgG in swine that were in close contact with each other was found to be significantly higher than in swine from different geographical regions (28.03% vs 15.10%, $P<0.0001$), which suggests that the transmission of swine HEV may be associated with close contact among swine within a particular herd.

The data on anti-HEV in swine reported here indicated that swine may serve as a reservoir for certain strains of HEV in South Africa, as has been suggested for other parts of the world, such as Canada and Korea (32). In terms of observations elsewhere, it would appear that these HEV strains, or mutants of these strains, might cause sub-clinical infections in humans, eliciting an anti-HEV response (19, 28, 32, 39). This could explain the detection of HEV antibodies in seroprevalence studies. Although HEV seems to be endemic in certain areas of South Africa, detailed genetic characterisation and epidemiological analysis of HEV strains, circulating among humans and swine in the

country, may cast light on the absence of clinical cases of hepatitis E. The relatively high prevalence (17.4%) of anti-HEV in communities living under conditions of poor hygiene and lack of sanitation (34) suggested that water may play a role in the transmission of HEV strains among humans, or from swine to humans (12).

Acknowledgements

We would like to thank Dr PJ Becker from the Biostatistics Unit of the Medical Research Council, Pretoria, South Africa for his contribution to the statistical analyses. This project was supported by Grant 02/18 from the Poliomyelitis Research Foundation, and financial support by the Water Research Commission.

3.6 References

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Table 3.1 Prevalence of anti-HEV IgG in swine from the Gauteng and Limpopo Provinces of South Africa

Sample Origin	No*	% Pos†	95% CI‡
Limpopo Province	101	15.84	(14.85-16.83%)
Gauteng Province	91	14.29	(13.25-15.33%)
TOTAL	**192**	**15.10**	**(14.39-15.81%)**
Warmbaths, Limpopo Province§	132	28.03	(27.17-28.89%)

*Number of serum specimens tested

†Percentage seropositive

‡95% Confidence interval

§Serum specimens of swine from one farm in the Warmbaths District, Limpopo Province